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Review

eIF4E phosphorylation regulates ongoing pain, independently of inflammation, and hyperalgesic priming in the mouse CFA model

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ABSTRACT

Mitogen activated protein kinase-interacting kinase (MNK)-mediated phosphorylation of the mRNA cap binding protein eIF4E controls the translation of a subset of mRNAs that are involved in neuronal and immune plasticity. MNK-eIF4E signaling plays a crucial role in the response of nociceptors to injury and/or inflammatory mediators. This signaling pathway controls changes in excitability that drive acute pain sensitization as well as the translation of mRNAs, such as brain-derived neurotrophic factor (BDNF), that enhance plasticity between dorsal root ganglion (DRG) nociceptors and second order neurons in the spinal dorsal horn. However, since MNK-eIF4E signaling also regulates immune responses, we sought to assess whether decreased pain responses are coupled to decreased inflammatory responses in mice lacking MNK-eIF4E signaling. Our results show that while inflammation resolves more quickly in mice lacking MNK-eIF4E signaling, peak inflammatory responses measured with infrared imaging are not altered in the absence of this signaling pathway even though pain responses are significantly decreased. We also find that inflammation fails to produce hyperalgesic priming, a model for the transition to a chronic pain state, in mice lacking MNK-eIF4E signaling. We conclude that MNK-eIF4E signaling is a critical signaling pathway for the generation of nociceptive plasticity leading to acute pain responses to inflammation and the development of hyperalgesic priming.

Introduction

New treatments are needed for the treatment of pain that simultaneously attenuate acute pain and prevent the development of chronic pain after injury (Skolnick and Volkow, 2016; Price and Gold, 2017; Volkow and Collins, 2017). Multiple lines of evidence support the contention that changes in nociceptor excitability that drive acute pain and lead to the development of chronic pain are dependent on activity-dependent signaling to translation machinery leading to the synthesis of new proteins that are critical for this form of neuronal plasticity (Khoutorsky and Price, 2018). We have recently focused on the mitogen activated protein kinase (MAPK) interacting kinase (MNK) family (the MNK1 and MNK2 kinases) (Moy et al., 2017; Khoutorsky and Price, 2018; Moy et al., 2018) that are the only known kinases that phosphorylate the mRNA cap-binding protein eukaryotic initiation factor (eIF) 4E (eIF4E) (Waskiewicz et al., 1999). While the entire repertoire of mRNAs whose translation is regulated by eIF4E phosphorylation is not known, eIF4E phosphorylation regulates the translation of a number of mRNA that are involved in nociceptive plasticity, such as matrix metalloprotease 9 (MMP9) (Gkogkas et al., 2014) and brain-derived neurotrophic factor (BDNF) (Moy et al., 2018), in addition to many cytokines and chemokines (Furic et al., 2010; Silva Amorim et al., 2018) that are involved in pain signaling. Mice that harbor a deletion at the MNK1/2 phosphorylation site on eIF4E (*eIF4E*^{S209A} mice) have deficits in development of nociceptive behavioral plasticity in response to many inflammatory mediators, fail to show increased nociceptor excitability in response to these mediators using electrophysiological measures, and have decreased cold hypersensitivity after nerve injury (Moy et al., 2017, 2018). These effects are recapitulated by genetic or pharmacological neutralization of MNK1/2.

Since eIF4E phosphorylation regulates translation of mRNA involved in nociceptive plasticity and inflammation, an open question is whether behavioral effects observed in *eIF4E*^{S209A} mice are due to a loss of nociceptor plasticity *in vivo* or a reduction in inflammation. One way to test this hypothesis is to use a strong inflammatory mediator, such as complete Freund's adjuvant (CFA), in wild-type (WT) and *eIF4E*^{S209A} mice and non-invasively examine inflammation and pain at the same time. In the experiments described here, we set out to address this question using infrared imaging to assess inflammation and spontaneous paw guarding to assess ongoing pain (Djouhri et al., 2006), both after CFA injection. Our hypothesis was that pain and inflammation would be dissociated in *eIF4E*^{S209A} mice demonstrating that decreased

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nociceptor sensitization is the key factor in behavioral phenotypes observed in these mice. Our findings are consistent with this hypothesis.

A second goal of our experimental design was to assess development of chronic pain after CFA injection in *eIF4E*^{S209A} mice using the hyperalgesic priming paradigm. This experimental design is used to model the transition to a chronic pain state where the animal becomes susceptible to a persistent pain state upon injection of a normally subthreshold inflammatory stimulus (Reichling and Levine, 2009; Reichling et al., 2013; Kandasamy and Price, 2015; Price and Inyang, 2015; Khoutorsky and Price, 2018). While we have previously shown that *eIF4E*^{S209A} mice fail to develop hyperalgesic priming in response to many inflammatory mediators (Moy et al., 2017), we have not previously tested these mice with CFA as the priming stimulus. Our results are consistent with previous observations that MNK-eIF4E signaling is a key signaling factor in the development of hyperalgesic priming.

Methods and materials

Animals

Mice were bred and raised on a 12-h light-dark cycle with lights on at 7:00 AM. Food and water were available *ad libitum* in their home cages. *eIF4E*^{S209A} mice on a C57BL/6 background were from the Sonenberg laboratory at McGill University (Furic et al., 2010). Both C57BL/6 (WT) and *eIF4E*^{S209A} mice were bred at The University of Texas at Dallas to produce experimental animals. Between 3 and 4 weeks old, mice were weaned and ear clipped to verify genotypes. All mice weighed between 20 and 25 g at the time of experimental use. The Institutional Animal Care and Use Committee at The University of Texas at Dallas approved all animal procedures.

Behavior

Male and female mice were habituated for approximately 1hr to acrylic behavior boxes prior to beginning experiments. Guarding scores were evaluated as described in Brennan et al. (1996), Djouhri et al. (2006), Xu and Brennan (2009) Hindpaw mechanical thresholds were determined by using the up-down method as described previously (Chaplan et al., 1994) using calibrated Von Frey filaments (Stoelting Company, Wood Dale, IL). A forward-looking infrared (FLIR) T650SC camera (Wilsonville, OR) was used for thermal imaging. At each time point two pictures were taken and the mean temperature in degrees Celsius across each paw was recorded (Megat et al., 2017; Barragan-Iglesias et al., 2018). The experimenters (*JKM, JLK, TAS-P, and GP)* were blinded to the genotype of the mice.

Chemicals

Complete Freund's Adjuvant (CFA) was purchased from Sigma-Aldrich (St. Louis, MO) at a concentration of 1 mg/mL. For hindpaw injections, CFA was mixed with an equal volume of 0.9% saline and vortexed to create an emulsion. The emulsion was vortexed prior to each injection (10 μL) to ensure equivalent injections between animals. Prostaglandin E_2 (PGE_2) was purchased from Cayman chemicals (Ann Arbor, MI). All other chemicals were attained from ThermoFisher Scientific (Waltham, MA).

Statistics

All data are shown as mean ± standard error of the mean (SEM), with individual samples represented within graphs to depict the n of each group and the distribution of the data points. GraphPad Prism 6 v 6.0 for Mac OS X was used for analysis. Statistical tests, post hoc analyses, and values for each figure are displayed in Table 1.

Results

CFA-induced inflammation and spontaneous pain are dissociated in *eIF4E*^{S209A} mice

To test if CFA-induced inflammatory responses are regulated by eIF4E phosphorylation, WT and *eIF4E*^{S209A} mice were injected with 5 μg of CFA and a FLIR camera was used to observe changes in temperature in the ipsilateral and contralateral hindpaws. FLIR imaging was used because it allows for non-invasive monitoring of temperature changes that can be paired with behavioral measures. The ipsilateral hindpaw in WT mice displayed a dramatic increase in temperature, indicative of inflammation, compared to the contralateral hindpaw starting at 3 h post CFA injection. This effect lasted for 7 days in WT mice (Fig. 1A & B). Similarly, the injected hindpaw in *eIF4E*^{S209A} mice showed an increase in temperature compared to the contralateral hindpaw at 3 h post CFA injection and this change persisted through the 72 h measurement (Fig. 1A & C). However, on Day 7, CFA-injected paws in WT mice had significantly higher temperatures compared to *eIF4E*^{S209A} mice (Fig. 1D) and ipsilateral hindpaws of *eIF4E*^{S209A} mice were not different from contralateral hindpaws on day 7 after CFA injection. While there is a late difference in inflammation induced by CFA in *eIF4E*^{S209A} mice, the early effects are indistinguishable from WT mice, at least with this measure.

CFA injection causes guarding behaviors in rodents that are linked to ongoing activity in nociceptors (Djouhri et al., 2006; Weibel et al., 2013). These ongoing pain behaviors are present at 24 h after CFA injection but subside rapidly after that, as does the ongoing activity in nociceptors (Djouhri et al., 2006). We hypothesized that ongoing pain would be decreased in *eIF4E*^{S209A} mice given our previous findings that CFA-induced mechanical hypersensitivity is decreased when the MNK-eIF4E pathway is disrupted (Moy et al., 2017). In line with this hypothesis, we observed significantly less guarding in *eIF4E*^{S209A} mice at 24 h after CFA injection (Fig. 1E). Therefore, even though there is no difference in signs of inflammation for at least the first 3 days after CFA injection, evoked and ongoing pain behaviors are reduced in mice where the MNK-eIF4E signaling pathway is disrupted. This strongly suggests that differences in pain behaviors observed when this pathway is disrupted are dependent on decreased nociceptor plasticity and not on decreased inflammatory responses. Male and female mice were used in these experiments and we did not note any sex differences, consistent with our previous work on MNK-eIF4E signaling.

CFA-induced hyperalgesic priming requires eIF4E phosphorylation

Previously, we found that eIF4E phosphorylation is required for the development of hyperalgesic priming using stimuli such as nerve growth factor (NGF), interleukin 6 (IL-6), and protease activated receptor type 2 (PAR2) activation (Moy et al., 2017). Moreover, in MNK1/2 double knockout mice CFA-induced hyperalgesic priming is reduced suggesting that eIF4E phosphorylation also plays a key role in this model. We evaluated hyperalgesic priming with CFA as a priming stimulus using WT and *eIF4E*^{S209A} mice with mechanical sensitivity, guarding and temperature changes as experimental endpoints.

First, we assessed whether PGE_2 also induces changes in hindpaw temperature in mice primed with CFA. Previously primed WT and *eIF4E*^{S209A} mice were injected with 100 ng of PGE_2 and hindpaw temperatures were measured at 3 and 24 h after injection (Fig. 2A). PGE_2 induced a transient increase in temperature at 3 h in WT mice (Fig. 2B; left), whereas, in *eIF4E*^{S209A} mice no changes were observed when comparing the ipsilateral to the contralateral paw (Fig. 2B; right). The ipsilateral paws of *eIF4E*^{S209A} mice displayed a significantly lower temperature compared to WT mice 3 h post PGE_2 (Fig. 2C).

We then assessed whether PGE_2 injection induces guarding behaviors in mice primed with CFA. We observed guarding behavior elicited by PGE_2 injection in WT mice at 3 and 24 h post PGE_2 injection